LabChip XT DNA Kit, Assay User Guide

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Introduction

Applications

Sizing nucleic acid fragments is a time consuming, but critical part of many library construction protocols. Traditional, gel-based methods are notoriously inefficient and introduce variability that can negatively impact data quality. For paired-end and mate pair libraries, accurate size selection is critical, and improves assembly and facilitates the detection of structural variation. Non-sized libraries can be more difficult to assemble and often have errors in the detection of insertion and deletion events. Accurate sizing is also critical for removing artifacts introduced during the library construction process, such as adapter dimers. Separating fragments containing library from no insert fragments significantly improves the amount of data obtained from the sequencing reaction. The benefits of accurate and tight size selection include improved assembly of the sequenced data, less "non-align" reads, and increased average read length.

The LabChip XT/XTe fractionation system performs fast, automated nucleic acid sizing accurately and reproducibly. The XT/XTe replaces the traditional gel method and tightly sizes up to four samples in less than 60 minutes. Manual methods introduce run-to-run variability, resulting in less accurate sizing and variable collection widths. The LabChip XT/XTe provides improved collection efficiency (> 50%) and superior sensitivity, detecting less than 25ng of sheared sample. Fractionated samples are delivered in a sequencing compatible buffer. The XT improves laboratory efficiency and provides a level of sizing accuracy that is difficult to obtain using manual methods. Data is displayed digitally and non-fractionated sample can be recollected and used at another time.

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Features

- Fast separation time – separate up to 750bp in 30 minutes or less.
- Eliminates slab gels and manual excision to size select DNA.
- Automated sizing and quantitative information in a digital format.
- Included reagent set provides predictable, reproducible results.
- Resulting sample is tightly sized and is delivered in a sequencing compatible buffer.
- Size Selection may be performed using a software ladder, thus maximizing throughput.
- Each run can utilize 1-4 channels.
- Channel Operation Modes: Disabled, Ladder, eXtract and Stop, eXtract and Continue, eXcLuDe Region (XT only), Separation (XT only), eXtract and Pause, or Skip Extraction (XT only).
- Extraction Modes: size, fluorescence (XT only), peak start, peak max (XT only), smear max (XT only), or collect on click.

Specifications

LabChip XT DNA 750

DNA size range	***50 to 750 bp*** **Up to 1000bp with reduced resolution*
Minimum size distribution as expressed by CV	***5% at 300 bp***
Maximum load capacity	***1 µg of sheared genomic DNA, 50ng per fragment***
Minimum load capacity for optical detection	***25ng sheared genomic DNA, 0.5ng per fragment***
Sample Recovery	***>50%***
Time to 750 bp	***<30 minutes***

LabChip XT DNA 300

DNA size range	***25 to 500 bp***
Minimum size distribution as expressed by CV	***5% between 25 and 500 bp***
Maximum load capacity	***1 µg of sheared genomic DNA, 50ng per fragment***
Minimum load capacity for optical detection	***25ng sheared genomic DNA, 0.5ng per fragment***
Sample Recovery	***50-90%***
Time to 300 bp	***<45 minutes***

Kit Contents

LabChip XT DNA 750 Assay Kit: part number 760541

Box 1
- XT DNA 1K Ladder (yellow vial)
- XT DNA 6x Sample Buffer (green vial)
- XT DNA Collection Buffer (red vial)
- XT DNA Dye (blue vial)
- XT DNA Stacking Buffer (pink/purple vial)
- Store at 4ºC

Box 2
- LabChip XT DNA 750 Chip Kit (5 Chips)
- Store between 18-28ºC

LabChip XT DNA 300 Assay Kit: part number 760601

Box 1
- XT DNA 500 Ladder (yellow vial)
- XT DNA 6x Sample Buffer (green vial)
- XT DNA Collection Buffer (red vial)
- XT DNA Dye (blue vial)
- XT DNA Stacking Buffer (pink/purple vial)
- Store at 4ºC

Box 2
- LabChip XT DNA 300 Chip Kit (5 Chips)
- Store between 18-28ºC

Additional Items Required

- LabChip XT/XTe instrument with LabChip XT software version 2.0-SP1 or greater may be downloaded from http://www.caliperls.com/support/software-downloads.htm
- Buffer for dilution of ladder: EB (10mM Tris-Cl; pH 8-8.5) or TE Buffer (10mM Tris-Cl, 1mM EDTA; pH 8-8.5)
- Microcentrifuge tubes for sample preparation

Safety Warning & Precautions

! WARNING! For Research Use Only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

CAUTION We recommend that this product and components be handled only by those who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses, and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

! WARNING! Dye vial contains DMSO. Kit dye interacts with DNA and care should be taken to avoid inhalation and contact with skin and eyes.

Sample Preparation Procedure

The sample can be prepared up to 1 hour in advance of chip run, either before or after the creation of the run file. Caution: 6x Sample Buffer is light sensitive. Care should be taken to reduce light exposure of prepared sample.

1. Allow Reagent Kit to equilibrate to room temperature before use (approximately 20 to 30 minutes).
2. Ladder preparation
    a. Dilute 2µL of ladder (●) with 8µL of EB or TE Buffer (Use the same diluent as the sample buffer).
    b. Add 2µL of 6X Sample Buffer (●).
    c. Vortex sample to mix. Spin down.
3. DNA Sample preparation
    a. If necessary, adjust volume of DNA sample to 10µL by diluting with EB or TE.
    b. Mix 10µL of DNA sample with 2µL of 6X Sample buffer (●).
    c. Vortex sample to mix. Spin down.

Create LabChip Run File

This step can be prepared in advance of sample preparation and chip preparation. The creation of the run file is not time sensitive.

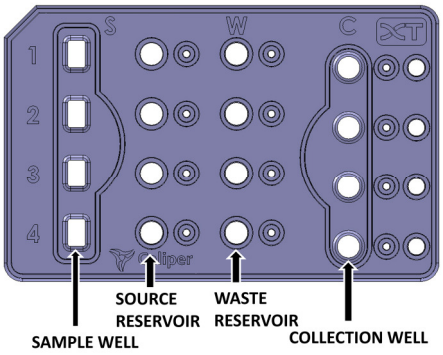
1. Launch LabChip XT/XTe software. On the LabChip XT/XTe Main Window, select **Tools →Run File Editor**. The Run File Editor window opens.
2. Select the Method (XT DNA 750, or XT DNA 300) to edit from the **Fractionation Method** drop down list. Each Method is restricted to a type of chip (ie DNA 300 or 750).
3. Under the Setup Tab, type the desired name for each channel in the **Sample Name** box.
4. For each channel, select the desired operation for the channel using the pull-down menu. Refer to the XT/XTe Instrument Manual for operations modes available for your instrument.
    a. Disabled - The channel will not be used in the run. Any channel that has already been used is automatically set to Disabled and cannot be reused.
    b. Ladder - The channel contains a ladder that is used to calculate the sizes in the samples in the remaining channels during the run.
    c. eXtract and Stop - The sample in the channel will run until the extraction is complete. After the extraction, no additional sample will move through the channel.
    d. eXtract and Continue - The sample in the channel will run until the end of the chip range. The size range specified in the Extraction Region will be collected in the collection well.
    e. eXcLuDe Region - The sample in the channel will be collected in the waste well on the chip except for the selected region, which moves into the collection well on the chip. The desired output is extracted from the waste well.
    f. Separation - The sample in the channel will run until the end of the chip range. No collection will occur.
    g. eXtract and Pause - Used for multiple extractions, the sample in the channel will be collected in the collection well and the run will pause for collection before resuming the run.
    h. Skip Extraction - Skips over a known region in the sample without extracting any sample to the Collection well. The software automatically moves to the next step in a multistep extraction immediately after the skipped region is complete. When used for multiple extractions, the first step of the extraction is set to Skip Extraction, typically using Peak Max as the extraction mode. The software will automatically identify the peak within the Search

Region, and prevent the next step from triggering through the size range designated by the Collection Width. The peak identified in the Skip Extraction step will be marked in grey on the electropherogram. The second step, typically Extract and Pause for the peak of interest, may be set with an overlapping Search Region.

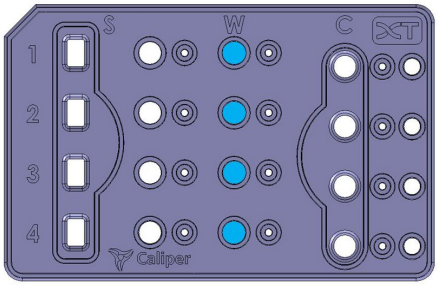
5. For each channel, select desired extraction mode using the radio buttons. With the exception of Size Range, the collection Width can be specified as the total number of base pairs (BP), a percent of the size at which extraction is centered (+/-%), or to be trigger by a Manual click.
    a. **Size Range** - The specified size range will be diverted to the chip collection well. Size range can be specified on the Setup Tab as a Size ± a percent of the size, or on the Advanced Tab as a specific start and end size in the Extraction Region text boxes. The Setup Tab and Advanced Tab are synchronized to always specify the same settings.
    b. **Fluorescence** – Collection begins when the specified fluorescence Threshold, in RFU, is exceeded.
    c. **Peak Start** – Collection begins at the start of a peak, when the specified slope Threshold, in RFU/minute, is exceeded.
    d. **Peak Max** - Centered on the first local maximum in the specified region. The signal slope within the search region must exceed the specified slope threshold (RFU/min) to arm this trigger. The extraction is triggered when the signal has switched to a negative slope which meets or exceeds the specified threshold in magnitude. If the end of the search region is reached after the trigger has been armed but before the negative slope threshold has been achieved, the extraction will trigger at the end of the search region.
    e. **Collect On Click** – Collection is initiated by the user.
        i. **LabChip XT software version 2.1 and later**. To select the highlighted region, click on the word CLICK in the Run Setup section on the left side of the LabChip XT Main Window. A dialog window will prompt you to confirm or cancel, and the target region will continue to update until confirmed. Note that for some width settings, the target region may extend past the last visible data point on the graph. In this case, the highlighted region will end at the last visible data point and the dialog box will indicate at what time the target region ends. When manual mode is selected in the width setting, click on the word STOP in the Run Setup section to end the collection or exclusion.
        ii. **LabChip XT software version 2.0 SP3 and earlier**. The extraction trigger point is selected by clicking at the desired time. You must click on the word CLICK in the Run Setup section on the left side of the LabChip XT Main Window. A window displays the time until the collection starts. This is the time until the selected sample size reaches the switch point where it is diverted into the collection well. You can also choose to start collection immediately or cancel collection. Based on the start size, the software projects the median collection size and continues to collect material until stopped manually, reached total number of BP or percent Collection Width specified in the text box.
    f. **Smear Max** - In Smear Max mode, the software will search within the specified region for the point at which two consecutive conditions have been met: 1) the signal has reached or surpassed the specified threshold signal (RFU) and 2) the signal is decreasing after averaging over three consecutive data points.
6. For XT only, click the Sizing Table Tab in the Run File Editor window.
    a. To use the default Caliper ladder, select the **Method Ladder** option.
    b. To use a custom ladder by specifying the sizes of the peaks in the ladder, select the **Custom Ladder** option and type the ladder peak sizes into the size column of the ladder table.
7. Click on Output tab and confirm Data Path and input the File Prefix/Project Name.
8. Click the Save button and save the created run file to the desired directory.

Prepare XT DNA Chip

Note: The dye contains DMSO and must be thawed completely before use. Gently vortex and centrifuge all kit reagents before use.



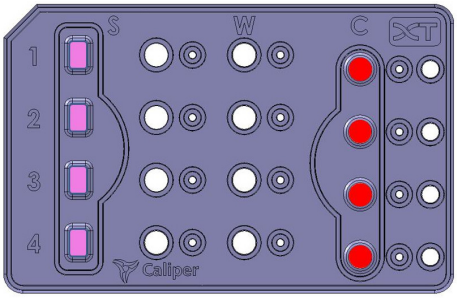
1. Remove Chip from foil bag and peel back top seal from chip.
2. Gently remove Sample Well and Collection Well combs.
3. If gel or buffer is present in either the sample or collection wells, it should be manually removed before sample loading. See "Chip Well Aspiration Using a Vacuum or Manual Pipette" at end of User Guide for illustrations.
4. If a well fills with buffer after vacuuming, do not use that channel.
5. If necessary, up to 60 uL of buffer for rinsing and/or collection can be taken from either the Source Reservoir or the Waste reservoir before the run and addition of dye.
6. Vortex the Dye and spin it down. Pipette 15µL of the XT DNA Dye (●) to the bottom of each of the four Waste Reservoirs.
7. Gently rock the chip to mix the dye and buffer. Visually check to see that the dye has been evenly distributed throughout the reservoir.
8. Ensure the top surface of the chip is dry.



ADD DYE

Steps 9-11 are time-sensitive steps. The run should be started within 5 minutes to prevent diffusion of loaded DNA sample and stacking buffer.

9. Add 20µL of the Collection Buffer to round Collection Wells (●).
10. Add 20µL of the Stacking Buffer to rectangular Sample Wells (●). Ensure that no bubbles remain in the well before proceeding to the next step.
11. Load the entire 12µL of the sample or prepared ladder into Sample Wells by layering under the Stacking Buffer. Position the pipette so that the tip gently touches but is not sealed against the bottom of the well. Pipette sample very slowly. The goal is to place the sample at the bottom of the well and to avoid mixing the sample with the stacking buffer.



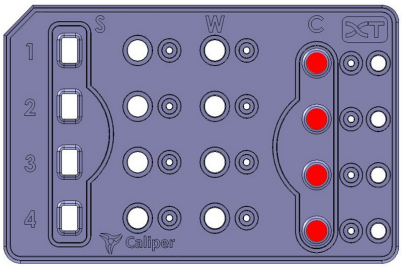
ADD COLLECTION & STACKING BUFFER

For best results, start the XT run within 5 minutes of loading the samples into the wells of the chip.

Starting the LabChip XT

1. Place the chip in the LabChip XT instrument with the positioning feature in the upper left corner aligning with the corresponding pattern on the instrument.
2. Close lid and select Instrument -> Start Run (or move the mouse over the instrument icon and click on the green run arrow).
3. To import the settings from a previously saved run file, click the Import Settings button at the bottom of the Start Fractionation window.
4. Select the desired run file, click the Open button and then click the Run button.
5. See LabChip XT User Manual or Run File Guide for more details on run parameter selection.

Collect Fractionated Material



COLLECT FRACTIONATED MATERIAL

For multiple extractions: During the pause after each collection, open the LabChip XT instrument lid and pipette recovered DNA sample (●) from each collection well into a clean tube for downstream processing. If necessary, wash well with 20µl buffer and add 20µl buffer for next collection. Close lid and click Resume.

For single extractions: After run has completed, open the LabChip XT instrument lid and pipette recovered DNA sample (●) from each collection well into a clean tube for downstream processing.

Analyzing Data

Refer to the LabChip XT User Manual for Data Analysis.

Typical Results

Example 1: Ladder and 3 channels with DNA of varying mass input and size selection (eXtract and Stop mode) XT 750

1. Channel 1: Caliper sizing ladder
2. Channel 2: 250ng input, select by size range: 600bp +/-5% (blue)
3. Channel 3:100ng input, select by size range: 300bp +/-5% (red)
4. Channel 4: 1000ng input, select by size range: 100bp +/-5% (brown)

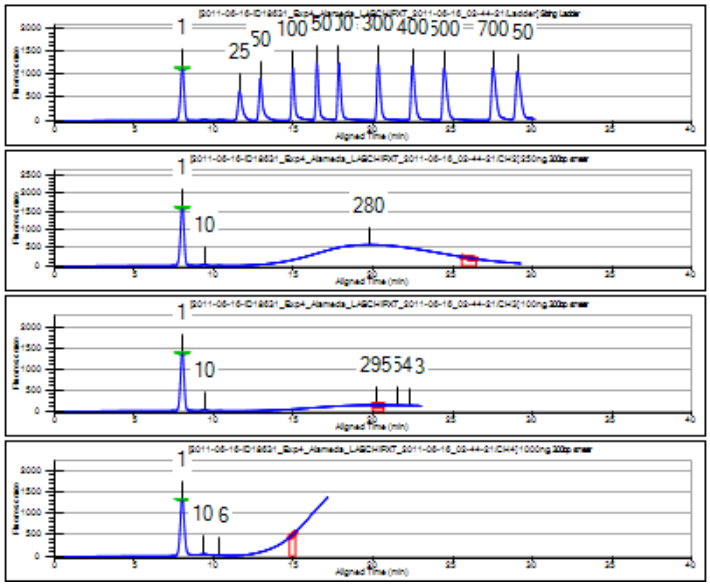


Figure 1A. LabChip XT Electropherogram

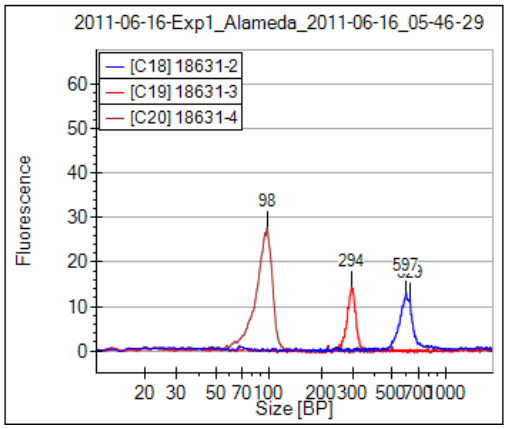


Figure 1B. LabChip GX Electropherogram of collected material

Example 2: Ladder reproducibility within one chip (separation only mode) 220ng of ladder in each channel. XT 750

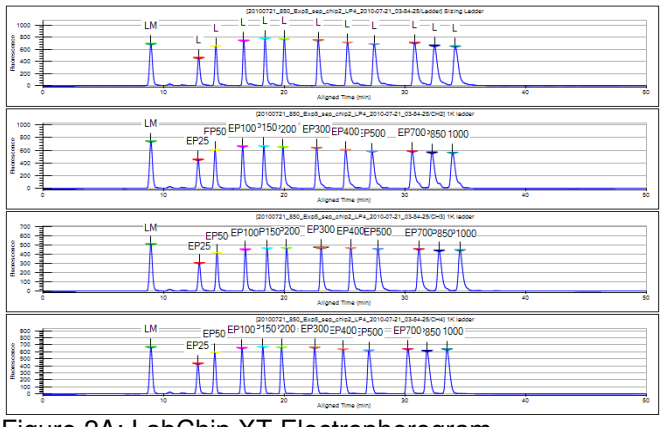


Figure 2A: LabChip XT Electropherogram

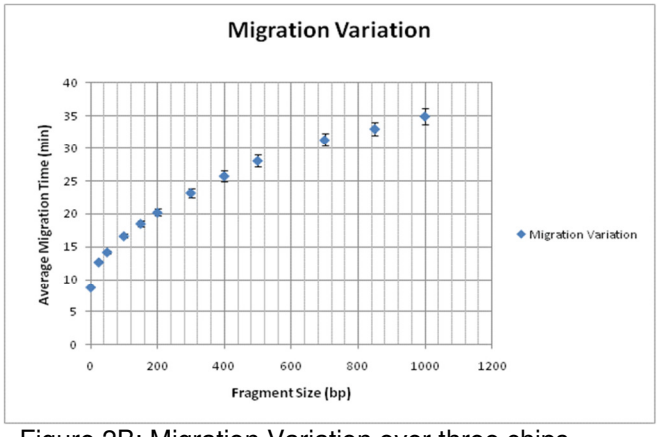


Figure 2B: Migration Variation over three chips

Example 3: Advanced Extraction Modes. 250ng of DNA, XT 300

1. Channel 2: 500ng input
   Step- 1) Skip Extraction, Peak Start: Threshold at 150RF/min, Width at 30bp (grey)
   Step- 2) Extract and Stop, Peak start: Threshold at 150RFU/min, Width at ±10% (red)

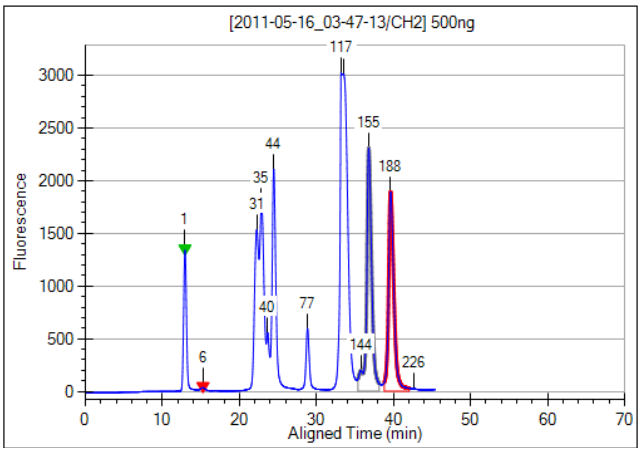


Figure 3A. LabChip XT Electropherogram

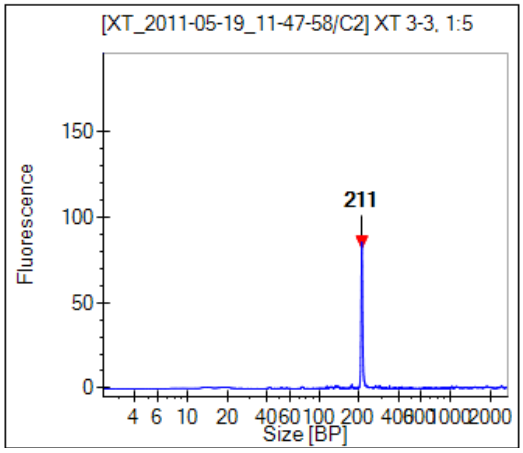


Figure 3B. LabChip GX Electropherogram of collected material

Example 4: Multiple Extractions across 400-600 bp Region (eXtract and Pause mode) XT 750

1. Channel 1: Caliper sizing ladder
2. Channel 2: 100ng input, select by size range:
   Step- 1) Extract and Pause, 430bp ±7% (blue)
   Step- 2) Extract and Pause, 500bp ±7% (red)
   Step- 3) Extract and Pause, 568bp ±6% (brown)

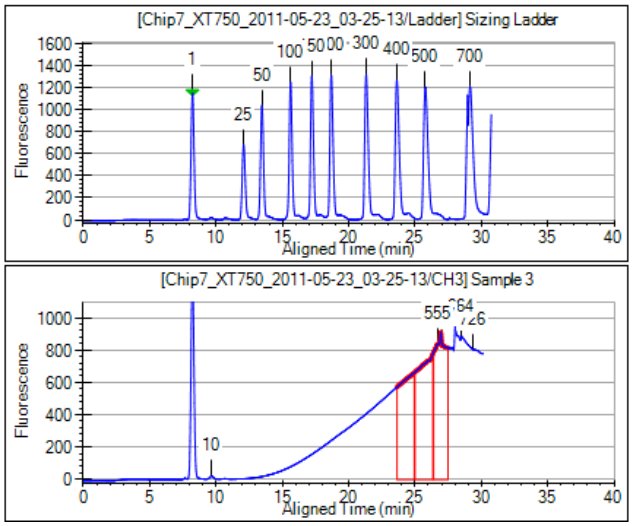


Figure 4A. LabChip XT Electropherogram

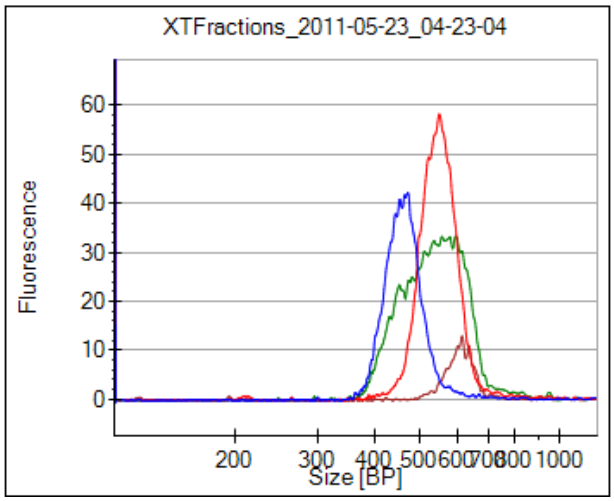


Figure 4B. LabChip GX Electropherogram of collected material. The green trace represents the pooled collections.

Example 5: Discrete Extractions at 180 and 400 bp (eXtract and Pause mode) XT 750

1. Channel 1: Caliper sizing ladder
2. Channel 2: 100ng input, select by size range: 180, 400bp +/-5% (blue, red)

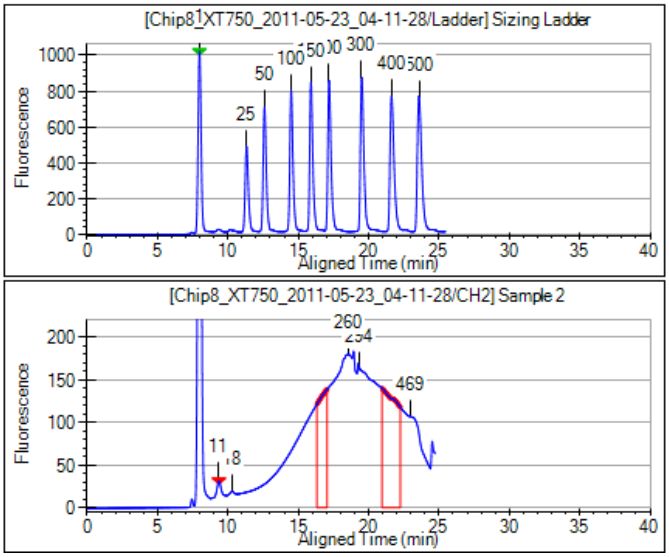


Figure 5A. LabChip XT Electropherogram

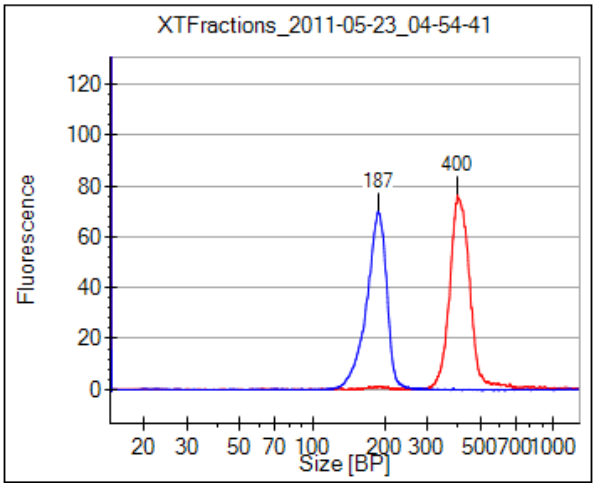


Figure 5B. LabChip GX Electropherogram of collected material

Example 6: Extraction of miRNA library (Skip Extract and eXtract and Pause modes) XT300

1. Channel 1: Caliper sizing ladder
2. Channel 2: 100ng miRNA library input,
   Step- 1) Skip Extraction, Peak Max: Threshold at 25RFU/min, Width at 25bp (grey)
   Step- 2) Extract and Pause, Peak start: Threshold at 50RFU/min, Width at ±5% (red)

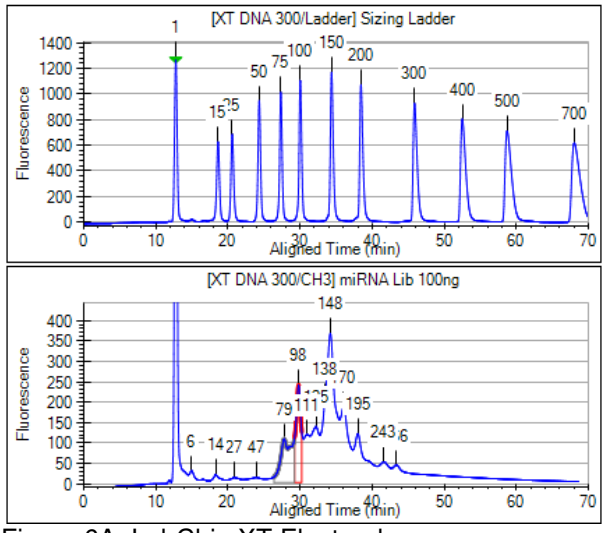


Figure 6A: LabChip XT Electropherogram

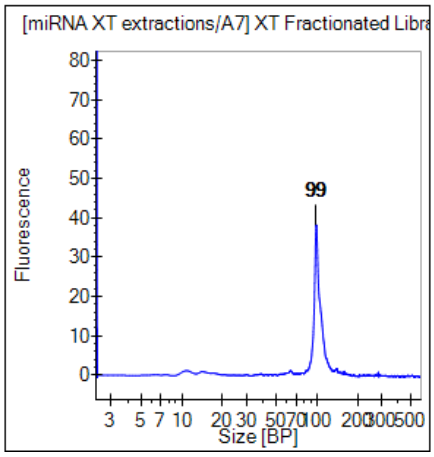


Figure 6B: GX electropherogram of collected material

Troubleshooting

- ***Chip fails connectivity test.***
  - Once a chip has failed a connectivity test, record which channel has failed. Remove the chip from the instrument.
    - Check that the outlets leading from the chip to the buffer reservoirs are not blocked by an air bubble. Air bubbles may be dislodged by gently tapping the chip near the location of the air bubble.
    - Check to see that 20µL of collection buffer and stacking buffer/sample have been added to their respective wells. Collection buffer is required in all channels used, even if no collection will take place.
    - Check to see that the surface of the chip and the underside of the instrument lid are free of buffer. If any buffer is found, remove buffer before beginning run again. If it is observed that the reservoirs do not have sufficient levels of buffer to cover the chip electrodes, the chip is defective; please contact Technical Support for chip replacement.
    - Note: if directed by Caliper Technical Support, it is possible to run the connectivity test separate from starting a chip run. See the description of the Test Chip button on the Start Fractionation Window in the LabChip XT User Manual.

- ***Lower Marker (LM) was not detected.***
  - An internal standard (LM) is added to samples to assist in determining size and concentration of the sample. The marker is also the first peak in the ladder. The lower marker is contained in the XT DNA 6x Sample Buffer (green vial).
    - If the lower marker is not detected, the marker might have degraded over time due to exposure to light. Repeat run again with fresh vial of 6x Sample Buffer.
    - A second possibility is that insufficient amount of 6x Sample Buffer was added to the sample. Ensure that the pipette is accurately dispensing 2µL of XT DNA 6x Sample Buffer.

- ***Ladder was not detected.***
  - Check that the optical windows on the XT instrument are not blocked or contaminated with any potentially fluorescent material, such as paper dust, dyes, reagents, or materials that may block light. Contaminants must be removed before starting a run.
    - Dust can be removed with pressurized and filtered air. If using a disposable pressurized air can, hold the can upright to avoid spraying propellant onto the optics.
    - Use a lint free wipe or swab dampened with deionized water to rinse the chip locator.
    - Allow all dampened surfaces to air dry.
    - Do not use detergents or other cleaners, as they may contain fluorescent dyes that interfere with the run.
  - Sample may not have been properly loaded into the sample well. If there are unused channels on the chip, repeat ladder load on a different channel and if the problem persists, repeat with a fresh vial of ladder.
  - Ensure that the sample diluent used with the DNA ladder (2µL 6x Sample buffer + 2µL ladder + 8µL sample diluent) does not contain high amounts of salt. This will reduce the stacking of DNA during separation. The recommended sample diluent is TE or EB; pH 8.0-8.5.

Ladder peaks are too broad.

- Broad or non-Gaussian ladder peaks are most commonly the result of poor sample loading. Ensure that the DNA sample is not being mixed with stacking buffer and that the sample is being loaded as described in step 12 of the section "Prepare XT DNA Chip" above.
- Gel in the sample well (not completely removed by the combs) can also cause broad ladder peaks. Ensure that all agarose has been removed from the sample wells without disrupting the gel in the channel. See "Chip Well Aspiration Using a Vacuum or Manual Pipette" at the end of this guide. Ensure that the stacking buffer (pink vial) was used in the sample well and not another reagent. Without the stacking buffer, the peaks will appear broad.
- Ensure that the sample diluent being used with the DNA ladder (2µL 6x Sample buffer + 2µL ladder + 8µL sample diluent) does not contain high amounts of salt. This will reduce the stacking of DNA during separation. The recommended sample diluent is EB or TE, pH 8.0-8.5.

- ***Ladder has double peaks.***
  - Sample may not have been properly loaded into the sample well or there may have been excessive gel in the sample well. Ensure that all agarose has been removed from the sample wells without disrupting the gel in the channel. See "Chip Well Aspiration Using a Vacuum or Manual Pipette" at the end of this guide. If there are unused channels on the chip, repeat ladder load on a different channel and if the problem persists, repeat run again with fresh vial of ladder.
  - Ensure that the sample diluent being used with the DNA ladder (2µL 6x Sample buffer + 2µL ladder + 8µL sample diluent) does not contain high amounts of salt. This will reduce the stacking of DNA during separation. The recommended sample diluent is EB or TE; pH 8.0-8.5.

- ***During the run, the ladder peaks suddenly appear broad or slow down in migration.***
  - Ensure that there is a sufficient amount of buffer in the reservoirs.
  - Ensure that the dye is mixed uniformly throughout the buffer waste reservoir. Non-uniformity across a reservoir may contribute to variation in sample migration within the separation channel.
  - Check for buffer on the surface of the chip which may create a short between electrodes.
  - Ensure that the sample diluent used in the ladder does not contain high amounts of salt which can affect DNA migration and stacking.

- ***Size selected material was too large or too small.***
  - Non-homogeneity of dye within a reservoir can cause differences in migration. It is important that the dye is mixed well in the waste reservoir.
  - Ensure that the sample well has not been overloaded with DNA. To achieve the specified sizing accuracy, the sample load should not exceed 1000ng of sheared DNA as over-sizing can occur with overloading.
  - High amounts of salt can affect DNA migration and stacking, which may lead to inaccurate sizing. The sample is recommended to be in EB or TE; pH 8.0-8.5.

- ***Size selected material has shoulder on leading edge or trailing tail of peak when size selected material has been analyzed by LabChip microfluidic separation***
  - Shoulders or tails (non-Gaussian behavior) can occur when samples are not loaded properly. Ensure that the DNA sample is not being mixed with stacking buffer and that the sample is being loaded as described in step 12 of the section "Prepare XT DNA Chip" above.

- Secondary structure within the sample during separation can also lead to non-uniformity of fractionated material. To confirm that secondary structure within sample is the cause of non-uniform peaks, if feasible, try heating a sample post-extraction to 70ºC for a few minutes, snap cool by immersing sample in ice, then reanalyze. If DNA no longer has shoulder or tail, the irregular shape of the size selected material was an artifact of the analytical assay (Figure 7).

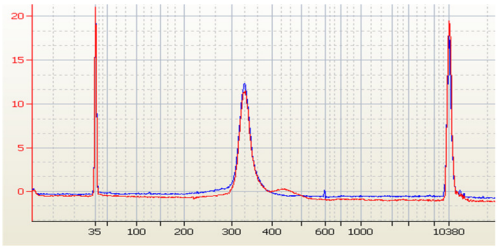


Figure 7. Secondary structure of fractionated sample (red). Sample heated to 70ºC for a few minutes ,snap cool and reanalyze (blue).

- ***Fluorescent signal is weak.***
  - Check that the optical window on the XT instrument is not blocked or contaminated with any potentially fluorescent material, such as paper dust, dyes, or reagents. Contaminants must be removed before starting a run.
    - Dust can be removed with pressurized and filtered air.
    - To clean spills, use a lint free wipe or swab dampened with 70% - Isopropanol solution in deionized water to clean the lid and chip locator.
  - Run an optics diagnostic test, under the Validation menu. If a channel's gain is not adequate or background is too high, contact Caliper Technical Support.

- ***Optical detection was saturated during run.***
  - Saturation may occur if more DNA is loaded than supported by the assay specifications.
  - Run an optics diagnostic test using the test chip, under the Validation menu. If any diagnostic failures are reported, contact Caliper Technical Support.

- ***Collection well was empty after run.***
  - Ensure that enough Collection Buffer was added to the collection well to completely fill the bottom of the well before the run has started. The failure to add collection buffer, however, should result in a failed connectivity test.
  - Chip may have been left on instrument too long and sample might have dried over time. It is important to recover the sample immediately after a run is completed to avoid loss of sample.
  - Laboratory temperature may be too high and sample is evaporating over the course of the run.
  - Incorrect reagent was used in the collection well. Ensure that the collection buffer (red vial) was used.

- ***Collection well has more buffer than was added at the beginning of the run.***
  - If the sieving material in the channel entering or exiting the collection well is disrupted, buffer can continuously flow into the collection well. See step 6 in the section "Prepare XT DNA Chip" above. If buffer is flowing into the collection well, the channel is considered to be compromised and should not be used.

- ***Top of chip was wet with buffer after run.***
  - Ensure that the top of the chip is dry before beginning the run
  - Buffer may form a liquid bridge in the openings to the buffer reservoirs during mixing of the dye. Typically any meniscuses in these openings will break on their own within a few minutes. Occasionally liquid in the meniscus will be transferred to the surface of the chip during a run. Generally this has no impact, but this occurrence can be eliminated by using a pipette tip to remove any meniscus of liquid in the openings to the reservoirs on the top surface of the chip prior to starting the run.

- Inspect electrodes for buffer. Use a lint free swab dampened with deionized water to rinse the electrodes if any buffer contamination is seen. Allow electrodes to air dry before using instrument.

- ***Sieving material in chip appears cracked or dislodged after opening from foil packaging.***
  - Temperature of the chip may have exceeded optimal recommended temperature during shipment or storage. Wide fluctuations in temperature may affect sieving material integrity.
  - Foil seal over chip may have been damaged during shipment. If a chip is not sealed properly, fluctuations in pressure may affect sieving material integrity.
  - Any affected channels should not be used.

- ***Chip leaks buffer from the bottom of the chip.***
  - Chip was damaged during handling or shipment, contact Caliper Technical Support.
  - Note that once the seal is removed, the chip should not be inverted as buffer can flow out of the reservoir openings in the top of the chip. Such leakage would be expected and does not indicate a defective chip.

- ***A buffer reservoir is empty after opening foil packaging.***
  - The chip is defective, contact Caliper Technical Support.

- ***Sample well filled with buffer after comb removal or after excess sieving material was vacuumed from a well.***
  - If the sieving material in the channel is disrupted, buffer can continuously flow into the sample or collection well from surrounding reservoirs. This is a symptom of the sieving material having been compromised or dislodged in the channel and can lead to poor resolution.
  - If the sample well fills with buffer only after removing of excess gel by vacuum, take care not to form a strong vacuum seal at the bottom of the well, thereby disrupting or possibly removing gel from the channel
  - If the sample well fills with buffer immediately after comb removal and refills after buffer is removed, then the chip is defective, contact Caliper Technical Support.

Frequently Asked Questions

Kit Specification

1. **What is in the Ladder?**

   The XT DNA Ladders are a mixture of individually chromatography-purified double stranded DNA fragments. The ladder is recommended for sizing and approximate quantification of a wide range of double-stranded DNA. The ladder is supplied in Storage Buffer (TE buffer) which is made up of 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA. Ladder data is used to convert the migration time of each sample peak into a size.

2. **What is in the 6xSample Buffer?**

   The 6x Sample Buffer contains the lower marker (first peak in the ladder), DMSO and glycerol. The lower marker is not a nucleic acid and will not interfere with sequencing. Once the 6x Sample Buffer has been mixed with the sample of interest, glycerol increases the density of the DNA sample and ensures that it will load below the stacking buffer. It is imperative that the stacking buffer is not mixed with the DNA sample below to ensure proper stacking of the DNA.

3. **What is in the collection buffer and is it compatible with sequencing?**

   The collection buffer is formulated with a Tris-acetate based buffer containing a small amount of EDTA that is mixed with a polymer component which increases extraction efficiency. It is slightly basic in pH (8.3) and is compatible with sequencing and downstream analysis.

4. **What is the maximum amount of DNA that can be loaded in each channel?**

   The maximum amount of DNA that can be loaded into one channel is 1µg of sheared DNA (fragmented to a distribution within the kit's specifications, XT750: 50-750bp; XT300: 25-500bp) or 50ng of a single fragment double stranded DNA.

5. **What happens if I overload the chip with DNA?**

   Overloading a particular channel with more than 1µg of sheared DNA can result in poor sizing and/or resolution. Loading DNA beyond specifications may lead to under-quantification of the total mass, optical saturation, and an electropherogram that is not representative of the actual population of input DNA.

6. **What are the smallest and largest sizes that the XT can recover?**

   The XT DNA 750 (300) kit can recover DNA sizes centered from 50bp to 1000bp (25bp to 500bp).

7. **How can extraction width be measured?**

   For the Gaussian peaks that are typically extracted, the standard deviation of the peak can be described as FWHM*0.425, where FWHM is the full width at the high signal maximum. The CV can then be calculated as the standard deviation divided by the peak size (CV = 0.425*FWHM/size).

8. **What is the minimum width that can be collected?**

   For the tightest size selection, it is recommended a collection width of +/-5% be used. Extraction widths of the collected material at the +/-5% collection width setting will be <10%CV from 50bp-750bp for the XT750 Assay and (<7.5%CV from 25bp-500bp for the XT300 Assay. Extractions above 750bp will have slightly wider extraction widths, typically <15%CV. Collection widths below +/-5% will decrease recovery with very little decrease in the size distribution of the collected material. Based on the requirement of the experiment, the user can optimize the collection width to ensure the appropriate balance between a tight size selection and optimal recovery.

9. **What is the maximum width that can be collected?**

   The collection width can be increased to +/-15% to increase the amount of material recovered. Collection widths above +/-15% can result in over-sizing with only small increases in the amount of material recovered. Based on the requirement of the experiment, the user can optimize the collection width to ensure the appropriate balance between a tight size selection and an optimal recovery.

10. **What are typical recovery rates of sample?**

    Typical recovery rates of DNA sample are 50%-90% when using the eXtract and Stop or eXtract and Pause mode.

11. **Can I load DNA smear in all four channels and size select without the use of a ladder**?

    Yes. The Sizing Table Tab in the Start Fractionation Window specifies the ladder to use when aligning all the sample channels on the chip. A user has three choices for a ladder.
    - Use the Caliper ladder included in the kit in a channel on the chip. This allows the user to have three channels for size selection and one for the ladder.
    - Use the Caliper method ladder which is a virtual historical ladder based on the kit ladder provided. This allows the user to have four channels for size selection.
    - Use a custom ladder provided by the user. A custom ladder may be used as an alternative to the kit ladder when a user wants to have more peaks in a region of interest. This allows the user to have three channels for size selection and one for the ladder.

    The Sizing Table Tab contains the following settings:

    Method Ladder - If selected, the default Caliper-supplied ladder is used to align the samples during the run.
    Custom Ladder - If selected, the sizes in the Size Table as defined by the user are used.

    For optimal sizing accuracy when using the size range extraction mode, Caliper recommends using the Caliper supplied ladder. Optically assisted extraction modes often do not require the use of the supplied ladder.

12. **If I don't use all four channels during a run, can I use the remaining channels later?**

    Yes, but Caliper does not guarantee that the chip will comply with specifications of the kit when it is partially used. It is critical that the chip remains sealed when not in use to prevent drying of the gel matrix. For optimal performance Caliper recommends using all channels at once.

Assay Preparation

1. **How long can DNA remain in sample well after being loaded?**

   Caliper recommends that a user run the sample within 5 minutes of sample loading.

2. **How long can DNA remain in the collection well after being run?**

   Caliper recommends that a user collect the sample within 20 minutes of a run ending.

3. **Can I use my own buffer in the collection well?**

   Caliper does not recommend using alternate chemistries with XT reagents. Standard reagents have been carefully optimized for best performance.

4. **Can I use my own dye?**

   Caliper does not recommend using alternate dye chemistries with XT chips and reagents.

5. **Can chips or individual channels be reused?**

   Chips and individual channels cannot be re-used. Once a channel has been used, Caliper cannot guarantee that the chip will run properly or that there will not be any cross-contamination. The software automatically disables any channel that has previously been used to prevent accidental reuse.

6. **How do I maximize recovery of my DNA?**

   The best way to maximize DNA recovery is to stay within kit specifications (do not over-load channel with excess DNA, practice proper sample preparation/chip preparation/DNA loading techniques, homogeneous mixing of dye and buffer, and use low salt DNA diluent for maximum stacking). For maximum run speed and collection efficiency, choose the “extract and stop” operation or use “extract and pause” when collecting multiple fractions.

7. **What happens when buffer capacity is exceeded?**

   The pH and current will begin to drift when the buffer capacity is exceeded, eventually resulting in poor performance. There is no sudden drop in performance when the warning is issued, the limits are offered as a guideline that should give the most consistent results.

8. **How do I setup multiple extractions?**

   Refer to the LabChip XT Run File Guide.

9. **What are the recommended threshold settings for Peak Start and Peak Max for extracting small RNA library?**

   With some sample types, such as dsDNA libraries containing sequences derived from small RNA fragments, it is necessary to extract material that is very close in size to smaller unwanted material. The Skip Extraction mode allows the user to identify and exclude the peak of smaller unwanted material (containing no insert) from the adjacent peak containing larger fragments of interest. We recommend purifying the small RNA library prior to extraction.

For most libraries, in step 1, choose Skip Extraction for Operation, define Search Region for 50 - 200bp, choose Peak max for Trigger Mode and set Threshold for 5 -50RFU (we recommend 25RFU, lower thresholds may mistake baseline noise as a peak, higher thresholds may miss a peak), and set Collection Width for 25 - 30bp (depending on width of the adapter-dimer peak). Using Peak Max for Step 1 will center the skip region at the peak and is less sensitive to peak fronting. This is the recommended method. Peak Start can be used if the falling edge of the peak to be skipped is not sharp enough for the software to identify a maximum. (Peak Max requires both positive and negative slope thresholds to be exceeded; Peak Start only requires a positive slope threshold to be exceed). Smear Max may also be used when the expected height of the peak to be skipped is known. Increasing the skip collection width for Step 1 will delay the start of Step 2 (and should reduce carryover of the unwanted peak).

For step 2, choose Extract and Pause for Operation, define the Search Region for 50 – 200bp, choose Peak Start for the Trigger Mode and set the Threshold for 10 - 200 (we recommend 50RFU, higher threshold might miss the peak), and set Collection Width for 5 - 10% (depends on width of the library peak). Using Peak start for Step 2 minimizes the amount of possible carryover of the unwanted peak. Increasing width for Step 2 will increase the extraction time (but keep the same start) and may increase recovery.

10. Is a separate chip test required before the actual run?

No, it is not required but may be performed for troubleshooting purposes (e.g., when instructed by Caliper Technical Support). When running a separate chip test, put running buffer in the in the sample and collection wells before starting the test. You should not run this test with the sample loaded as the sample will be pushed into the channel during the test. After running the test you should carefully rinse the sample well and remove all liquid before proceeding with the normal chip preparation

11. What is the shelf life of the chips?

Chips are currently marked with a shelf life of 6 months from the date of manufacture. Customers are guaranteed a shelf life of 4 months from the shipping date.

12. Can I store the chips in the refrigerator to extend shelf-life of chips?

No, chips should be stored at room temperature (18-28ºC).

13. Can I load more than 10µL of sample but stay under 1µg per channel?

To achieve the stated kit specifications, 10 µL of sample is recommended. However, 15µL of sample per channel may be used with only a minor reduction in performance. This would require 3µL of 6x Sample Buffer per channel loaded (for a total of 18µL). It is important to keep the ratio of the sample buffer to customer sample the same.

14. How does one dispose of the chip after use?

Users should follow state/local/company/academic regulations for disposal of biohazardous medical wastes.

15. What happens if the ladder fails to be detected during a run?

If the software is unable to detect the real time ladder peaks, it will automatically default to the Caliper method ladder. This ladder is a virtual historical ladder based on the kit ladder provided.

16. **Can I recover sample from the waste reservoir?**

    Yes. DNA left in the waste reservoir can be recovered once a run has been completed. If the DNA is to be recovered from the waste reservoir, it is recommended that the user run an assay for the maximum allowable time (extract and continue or exclude) to ensure that the DNA of interest has entered the waste reservoir. Standard purification kits can be used to purify DNA from the collected waste reservoir.

17. **I see a scratch on the bottom of the chip. Will this interfere with optical detection?**

    Minor scratches on the bottom surface of the chip will not interfere with optical detection.

18. **My background level of fluorescence seems high. Can I clean the optical detection area?**

    Yes. See "Cleaning LabChip XT" in the user manual for a detailed description on how to clean the optical windows on the XT instrument.

19. **One channel seems to be running consistently faster than the other channels. Will this affect my size selection?**

    Some variation between channels is normal and migration times are normalized by the lower marker in each channel. Severe and consistent variations may indicate a problem with the instrument. Run the instrument diagnostics and report any failures to Caliper Technical Support.

Chip Well Aspiration Using a Vacuum or Manual Pipette

Removal of excess gel or buffer from the sample and collection wells can be accomplished by manual pipetting or by attaching a pipette tip to a house vacuum line or vacuum pump with trap (Figures 8A). Attach a pipette tip to the vacuum line and carefully aspirate excess gel. Caliper recommends using a pipette tip with an opening of approximately 1 mm and regulating the vacuum to no more than 20 in Hg to prevent too high a flow rate and possible dislodging of gel in the channel (Figure 8B). During vacuuming, avoid making a tight seal with the pipette tip at the bottom of the sample and collection wells as this could result in the removal of gel from the separation channel.

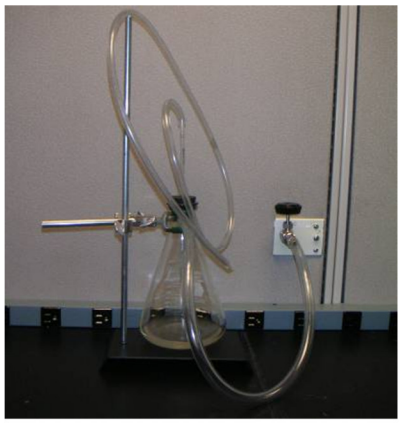


Figure 8A: Vacuum setup

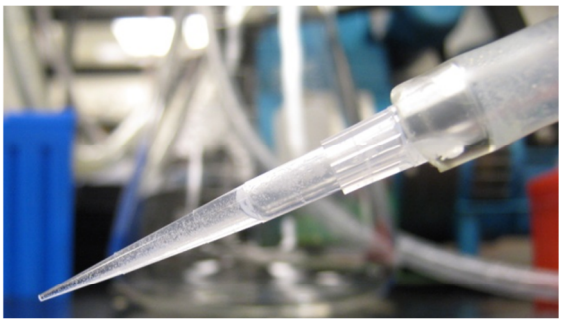


Figure 8B: Attached pipette tip for vacuuming of wells

To prevent contamination, use a new pipette tip over the permanent tip for each chip aspirated or a new pipet tip with each manual pipette. Ensure the well bottoms are not blocked by gel or buffer before proceeding (Figure 9).

Figure 9A. Sample well after comb removal (gel present)

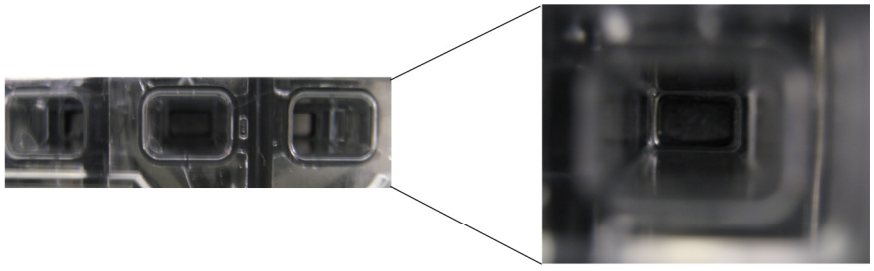
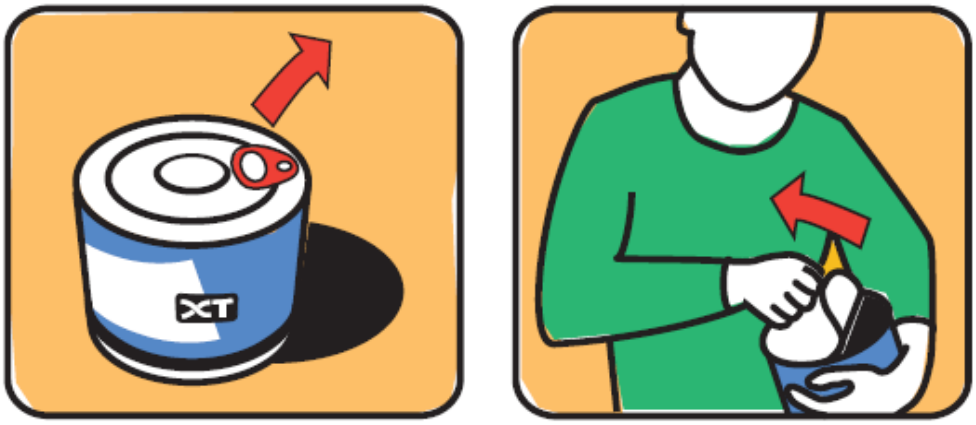


Figure 9B. Sample well after vacuuming (void of all gel)

68 Elm Street
Hopkinton, MA 01748-1668
1-877-LABCHIP (1-877-522-2447)

Opening Chip Container Manually



To manually open the chip container, first lift tab up until it is in a vertical position. Do not rock the tab back and forth. Next, position the can so that it is held securely in place with the smooth side of the can against your body. The tab should be positioned at 3 o'clock. Pull the lid back until it is detached. Carefully reach in to remove the chips.

Opening Chip Container with Optional Lid Opener

A lid opener is an optional accessory item (P/N 133524) that can be used to assist in opening of the XT chip containers. To use the lid opener, use the following steps:

Step 1: Open handles. Attach lid opener parallel to top of container aligning the two arrows. Be sure the cutting mechanism fits snugly against the side of the can. Close handles.
Step 2: Turn knob clockwise. After a full circle around the edge of container, you will feel the resistance drop.
Step 3: To release, turn knob slightly counter-clockwise and open handles.
Step 4: Use the Lid Gripper (small metal pliers on right side of can opener) to easily lift lid from container. Carefully reach in to remove the chips.

Reordering Information

Product	Part Number
LabChip XT DNA 750 Assay Kit	760541
LabChip XT DNA 300 Assay Kit	760601

Technical Support

Caliper Life Sciences
68 Elm Street
Hopkinton, MA 01748-1668
Phone: 1-877-LABCHIP (522-2447)
Fax: 1-508-435-3439

For additional assay and instrument troubleshooting, refer to the LabChip HT Software Help file. Call Caliper Technical Support at 1-877-LABCHIP.

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